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Large-volume injection in packed-capillary supercritical fluid chromatography

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Abstract

Injection of large sample volumes in packed-capillary supercritical fluid chromatography (SFC) with thermionic detection (TID) was performed using a packed precolumn set-up. The precolumn (2.0 × 1.0 mm I.D.) was loaded with an aqueous sample and dried with nitrogen at room temperature. After drying, desorption with a supercritical fluid to the analytical column was performed by switching restrictors mounted on valves. During desorption the analytes were focused on the top of the analytical column and next the analysis was started by again switching the restrictors. Parameters influencing the system, *e.g.*, desorption strength of the supercritical fluid, desorption volume and desorption flow-rate, were studied. By injecting large, *i.e.*, 75 μl, volumes of an aqueous sample, the analyte detectability was improved *ca.* 1000-fold compared with conventional 100-nl injections in hexane. This resulted in limits of detection of about 1 μg l^{-1} for three organophosphorus pesticides. The repeatability at the 5–15 μg l^{-1} level was better than 7% ($n = 8$), and good linearity over two orders of magnitude was found with spiked river water samples. To emphasize the potential of the system, a larger precolumn (10 × 2.0 mm I.D.) was tested, which was loaded with 47 ml of a river water sample. A further 1000-fold gain in sensitivity was obtained, and detection limits for the on-line trace enrichment–SFC–TID set-up now were in the 0.1 ng l^{-1} range.

1. Introduction

Packed-capillary supercritical fluid chromatography (SFC) can be used in combination with a wide variety of stationary phases and both liquid chromatographic (LC), *e.g.*, fluorescence or UV–Vis, and more sensitive and selective gas chromatographic (GC), *e.g.*, flame ionization or thermionic (TID) detection without the need to split the column effluent [1–3]. From economic and environmental points of view, other advantages of the technique are the small amounts of mobile phase needed and its low toxicity.

As regards injection, there are, however, still two important limitations. Sample introduction cannot be performed directly in the mobile phase and another medium has to be chosen for this purpose, usually an organic solvent such as hexane or acetone. The problem with the use of organic solvents for injection is that they are not always compatible with the selected detector. The second and more important limitation is that only relatively small injection volumes are possible, which results in unfavourable limits of

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detection in units of concentration. In the last 5 years, several techniques have been described that allow the injection of large volumes. Most of these techniques are based on time vent [4–6] and solvent backflush [7] or combinations of both [7] and have in common that they use apolar solvents (*e.g.*, hexane, chloroform) and were especially developed for capillary SFC. Recent reviews covering these developments have been given by Greibrokk and Berg [8], Koski and Lee [9] and Cortes [10]. Recently a new device has been described that is based on complete solvent elimination inside an inlet column followed by refocusing of the analytes on the top of a capillary column [11,12]. This system effects a 1000-fold increase in sensitivity; the repeatability is good and aqueous samples can be injected. Drawbacks of this system are that injection is performed at 100°C and that it is not possible to flush the capillary inlet column after injection in order to perform a clean-up, which may result in memory effects.

Both of the above problems can be overcome by coupling a packed LC precolumn on-line with a packed-capillary SFC system. The precolumn is used for both trace enrichment and sample clean-up. A relatively large volume of liquid sample is pumped through the precolumn, at room temperature, and the analytes are trapped. After clean-up, the precolumn is dried with nitrogen. Modified carbon dioxide can then be used for the desorption of the precolumn. Advantages of this set-up are the possibility of analysing aqueous samples on-line with SFC and of introducing large volumes, which will improve the limits of detection. Two approaches for the on-line coupling are possible. After loading and drying with nitrogen, the precolumn can either be simply switched between the SFC mobile phase delivery system and the analytical column with subsequent direct desorption and analysis, as was shown by Niessen *et al.* [13]. Alternatively, the precolumn can be desorbed with refocusing of the analytes on the top of the analytical column prior to analysis. This can be done by regulating the pressure of the mobile phase, which will strongly influence the solubility of the analytes. A high pressure in the precolumn results in a high desorption power whereas a low pressure in the analytical column will cause strong retention of the analytes. The different pressures can be obtained by properly switching restrictors on and off. The second approach allows one to reconcentrate the analytes on top of the analytical column and to regenerate and reload the precolumn for the next run while the previous sample is analysed.

In this paper, the second approach is discussed. Three organophosphorus pesticides (OPPs) of mutually different polarity were chosen to evaluate the performance of the LC–SFC–TID or, rather, the solid-phase extraction (SPE)–SFC–TID system. Parameters that are likely to affect the desorption efficiency, *viz.*, the strength of the desorption fluid, the desorption volume and the desorption flow, were studied. The first parameter is influenced by pressure, temperature and percentage of methanol. As it is experimentally complicated to vary the desorption temperature, the whole study was performed at room temperature. In other words, sorption and desorption of the analytes occurred at the same temperature. The effect of the desorption flow-rate on the desorption efficiency was studied by varying the dimensions of the restrictors. Variation of the desorption volume for several modifier percentages was studied in order to obtain maximum recovery. After optimization the technique was applied to the analysis of spiked river water samples.

2. Experimental

2.1. Direct injections

The system configuration is shown in Fig. 1A. A Phoenix-20 syringe pump (SFC pump in Fig. 1A) (Carlo Erba, Milan, Italy) was used for mobile phase delivery and pressure control. For direct SFC–TID analysis, valve 3 (Type EC10U; Valco, Schenkon, Switzerland) was switched to allow the mobile phase from the SFC pump to flow through the capillary between ports a and b and via valve 4 to the analytical column. Direct SFC injections were performed with valve 4, a

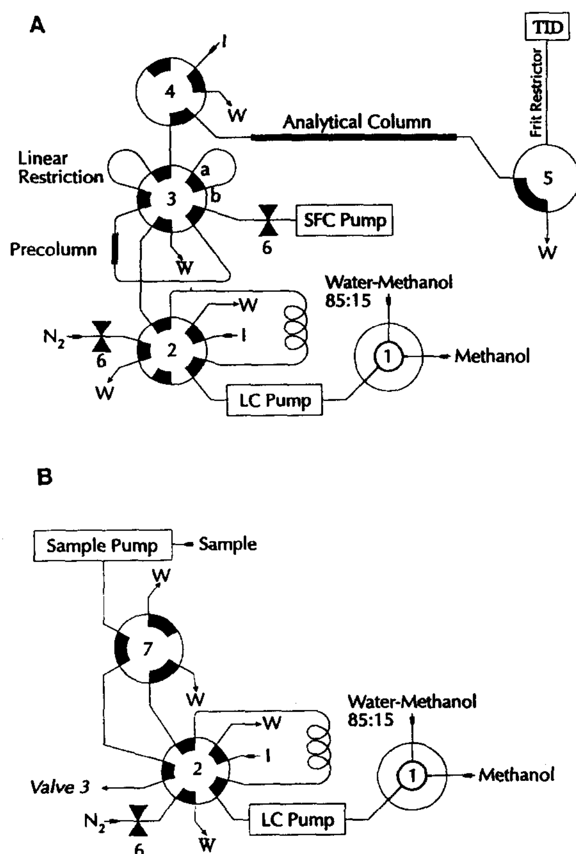

Fig. 1. (A) SPE–SFC–TID set-up. 1 = Solvent selection valve; 2 and 3 = ten-port switching valves; 4 = injection valve with internal 100-nl injection loop; 5 = three-port switching valve; 6 = on–off valve. The analysis and desorption can be performed with different methanol percentages in the mobile phase by connecting a second (analytical) pump and an on–off valve at port a of valve 3 and blocking port b with a stopper; the SFC pump then becomes the desorption pump. (B) Set-up for the introduction of large volumes with a sample pump. Valve 7 is a six-port switching valve. The set-up is connected to the SPE–SFC–TID set-up in (A) via valve 3.

100-nl Valco Type CI4W injection valve. The fused-silica column (135 × 0.32 mm I.D.) was slurry packed with LiChrosorb RP-18 (7 μm) bonded silica (Merck, Darmstadt, Germany). The column temperature was maintained at 50°C by a thermostated water-bath. Valve 5 (Valco Type EC3W) directed the column effluent through a 100-μm frit restrictor (Dionex/Lee Scientific, Salt Lake City, UT, USA) to the detector. The frit restrictor was used for pressure control and was shortened to give a flow-rate of 10 μl min^{-1} at 150 bar. The restrictor was situated in the detector base of a Carlo Erba NPD-40 thermionic detector as discussed by Verga [14]. After optimization as described by Mol *et al.* [15], the hydrogen and air flow-rates were set at 35 and 252 ml min^{-1}, respectively; the distance between the rubidium bead and the jet tip was 1.5 mm. The detector base was maintained at 300°C by an Ether type 17-90B heat controller. The thermionic detector was connected to a Model 180 electrometer (Carlo Erba) and for data acquisition a Model 4400 integrator (Varian, Walnut Creek, CA, USA) was used.

It was necessary to cool the syringe of the Phoenix-20 pump below 10°C during the filling procedure in order to obtain maximum filling percentages. This was done by slightly releasing the nut at the front of the syringe and allowing the carbon dioxide to expand adiabatically. A known volume of modifier (methanol) was added to the syringe. The resulting percentages (expressed as mol-% mol^{-1}) were calculated using interpolated carbon dioxide densities at various temperatures, taken from the tabulated data of Angus *et al.* [16], and the known densities of methanol. The SFC pump was allowed to stabilize overnight after filling. All analyses were performed using a pressure programme in which the mobile phase density was varied during a run [17].

2.2. LC–SFC–TID

A laboratory-made precolumn (2.0 × 1.0 mm I.D.) packed with Bondesil C_{18} (40 μm, preparative grade) material (Analytichem International, Harbor City, CA, USA) was used for trace enrichment. The sample solutions were prepared in water–methanol (85:15, v/v) and injected in a 75.1-μl injection loop on valve 2 (Valco Type EC10U). A Model 2150 LC pump (LKB, Bromma, Sweden) was used to load the sample on the precolumn with water–methanol (85:15, v/v) at a flow-rate of 0.10 ml min^{-1}. The same pump was used to condition the precolumn with water–methanol (85:15, v/v) at a flow-rate of 0.25 ml

min^{-1} for 5 min and to regenerate the precolumn with methanol at a flow-rate of 0.50 ml min^{-1} for 5 min. After loading of the sample, the precolumn was dried with nitrogen for 10–15 min at a flow-rate of 100 ml min^{-1}.

Desorption of the precolumn was performed by the simultaneous switching of valves 3 and 5 to the positions shown in Fig. 1A; the modified carbon dioxide was then directed through the precolumn. The linear restrictor (8.0–35.0 cm × 15 μm I.D.) (Polymicro Technologies, Phoenix, AZ, USA) between the precolumn and the analytical column was used to control the desorption flow-rate. After desorption with a certain volume, determined by the filling percentage reading of the SFC pump, valves 3 and 5 were simultaneously switched and the analysis was started using the same pressure programme as was used for direct SFC–TID.

In several series of experiments desorption was performed with a different modifier percentage to that used for the analysis. This was done by connecting a second SFC pump (Phoenix-20) and an on–off valve to port a of valve 3 and by blocking port b of that valve with a stopper (not shown in Fig. 1A). The first SFC pump and the SFC pump at port a were used for desorption and analysis, respectively.

Introduction of larger, *i.e.*, 3–50 ml, sample volumes was performed using the set-up shown in Fig. 1B. A Model 4140 LC pump (Kipp & Zonen, Delft, Netherlands) at a flow-rate of 0.3 ml min^{-1} was used. A laboratory-made six-port valve (valve 7) was used to switch from the sample to the water–methanol (85:15, v/v) used for conditioning and flushing the precolumn. The exact sample volume introduced was determined by calibration of the sample delivery flow-rate and measurement of the loading time. When using a larger (10 × 2.0 mm I.D.) precolumn, the same set-up as in Fig. 1B was used to introduce sample volumes of up to 50 ml at a flow-rate of 2.5 ml min^{-1}. Regeneration and conditioning were both performed for 6 min with a flow-rate of 2.5 ml min^{-1}. Drying of the precolumn was done with 100 ml min^{-1} of dry nitrogen for 30 min; 300 μl of modified carbon dioxide were used to desorb this precolumn at a pressure of 150 bar and a flow-rate of 60 μl min^{-1}.

2.3. Chemicals

Carbon dioxide (99.97%) and nitrogen (99.999%) were obtained from Hoek Loos (Schiedam, Netherlands), HPLC-grade hexane from J.T. Baker Chemicals (Deventer, Netherlands), HPLC-grade methanol from Rathburn (Walkerburn, UK) and diazinon (98%), pyrazophos (99%) and azinphos-methyl (99%) from Riedel-de Haën (Seelze, Germany). Solutions for direct SFC injections were made in hexane. River Meuse samples were taken at Keizersveer, Netherlands. To each 85 ml of sample, 15 ml of methanol were added, then the samples were filtered through a 0.2-μm Red Rim filter obtained from Schleicher & Schüll (Dassel, Germany).

3. Results and discussion

3.1. Preliminary tests

In order to be able to evaluate the performance of the SPE–SFC–TID system, first direct injections of the three OPPs used as model compounds were made in the SFC–TID system. The limits of detection for the pesticides varied from 30 to 100 pg (signal-to-noise ratio = 3) using 100-nl injections in hexane (Table 1). Hexane was used as this solvent has a less detrimental effect on the separation than acetone. Calibration plots from the limits of detection up to 4–12 ng injected were linear. The repeatability of the system for these pesticides varied from 3 to 11% ($n = 13$; injected on one day), and the reproducibility was 7–13% ($n = 8$; injected on two different days). The limits of detection expressed in units of concentration are of the order of 1 mg l^{-1}, which certainly is not sufficient for trace-level environmental studies. A second disadvantage is that the injection medium is hexane, which makes it necessary to perform a time-consuming sample treatment procedure to extract the analytes from an aqueous matrix. In addition, the more polar analytes can only be extracted by a more polar solvent, which will adversely affect the SFC separation.

Next, on-line SPE–SFC–TID was attempted

Table 1
Calibration data for direct injections of three organophosphorus pesticides in hexane

Analyte	Limit of detection[a] (mg l^{-1})	Largest amount injected (mg l^{-1})	Calibration graph[b]	
			$y = a(\sigma_a)x + b(\sigma_b)$[c]	R^2
Diazinon	0.3	40.0	$y = 135\,(2.7)x - 22.4\,(42.7)$	0.995
Pyrazophos	0.6	80.0	$y = 44.4\,(0.3)x - 42.7\,(56.5)$	1.000
Azinphos-methyl	1.0	120.0	$y = 31.3\,(0.5)x - 69.7\,(48.6)$	0.998

Column: 135 × 0.32 mm I.D. packed with 7-μm LiChrosorb RP-18. Mobile phase: carbon dioxide modified with 1.2 mol-% methanol at 50°C. Pressure programme: 0 min, 150 bar; 1 min, 150 bar; 6 min, 200 bar; 11 min, 210 bar; 14 min, 150 bar.
[a] Signal-to-noise ratio = 3.
[b] Ten data points in duplicate.
[c] Taken from limit of detection to largest amount injected.

by adding a short precolumn to the system (*cf.*, Fig. 1), which should trap the OPPs from aqueous samples. It was necessary to add some methanol to the sample to diminish the adsorption of the pesticides on the glass surfaces and the inner wall of the capillaries. No breakthrough was observed for these analytes after loading volumes of up to 10 ml containing 15 vol.-% of methanol. The procedure involves conditioning of the precolumn, loading of the aqueous sample on the precolumn and drying of the precolumn with nitrogen; these steps can all be implemented by switching valve 2 (Fig. 1A). Drying of the column material is necessary to prevent blockage of the linear restriction between the precolumn and the analytical column (valve 3, Fig. 1A) by water, which has a very high viscosity compared with modified carbon dioxide. A drying time of 10–15 min was selected as it was found to be long enough to achieve complete drying of the precolumn. Next, desorption was started by simultaneously switching valves 3 and 5. As a result, the modified carbon dioxide is led through the precolumn and the linear restrictor. The linear restrictor keeps the pressure through the precolumn high, which ensures efficient desorption of the analytes from the precolumn sorbent. As there is no restrictor at the end of the analytical column, there is a considerable pressure drop over the linear restriction between the precolumn and the analytical column. This drop in density provides the required strong retention of the analytes on the analytical column and as a result they become focused on the top of the analytical column during the desorption procedure. The analysis is then started by switching valves 3 and 5 and starting the pressure programme. By switching valve 5 the restrictor to the detector is switched in-line with the analytical column and separation and detection can take place, while the precolumn is switched to allow its simultaneous regeneration. In order to study the effects of several parameters on the desorption efficiency, a solution of the three OPPs was prepared in water–methanol (85:15, v/v). The concentrations were 5 μg l^{-1} for diazinon, 10 μg l^{-1} for pyrazophos and 15 μg l^{-1} for azinphos-methyl.

3.2. *Optimization of SFE–SFC–TID system*

The first parameter studied was the amount of methanol used to modify the carbon dioxide. Fig. 2 shows the effect of the modifier percentage on the desorption efficiency (expressed as relative TID response) of the analytes at 150 bar. The recovery of diazinon was low without methanol and rapidly increased when methanol was added, becoming maximum between 0.4 and 1.3 mol%. The other two compounds show similar plots but because they are more strongly retained by the precolumn packing material the optimum modifier percentages are 0.8 and 1.0 mol% for pyrazophos and azinphos-methyl, respectively. It is interesting to note the decreasing recovery at higher methanol percentages, which is caused by inefficient refocusing of the analytes

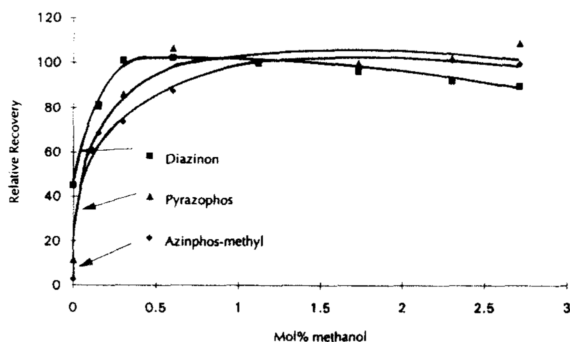

Fig. 2. Influence of the percentage of modifier on the recovery of (■) diazinon, (▲) pyrazophos and (◆) azinphos-methyl; response (peak area) for 1.1 mol% is 100%. Injected amount, 75.1 μl of 5 μg l^{-1} diazinon, 10 μg l^{-1} pyrazophos and 15 μg l^{-1} azinphos-methyl. All points are the means of at least six measurements; the relative standard deviations vary from 4 to 19%. Desorption with 300 μl of carbon dioxide modified with 1.1 mol% methanol at 150 bar and 60 μl min^{-1}. Precolumn: 2.0 × 1.0 mm I.D. packed with 40-μm Bondesil C_{18}. Analytical column: 135 × 0.32 mm I.D. packed with 7-μm LiChrosorb RP-18. Mobile phase: carbon dioxide modified with 1.2 mol% methanol at 50°C. Pressure programme: 0 min, 150 bar; 1 min, 150 bar; 6 min, 200 bar; 11 min, 210 bar; 14 min, 150 bar.

on the top of the analytical column. Because of this LC-type effect, the peaks are broadened and their integration becomes problematic, which results in lower peak areas.

Fig. 3 shows the influence of the desorption pressure on the desorption efficiency of pyrazophos for two modifier percentages. A higher methanol percentage is seen to have a more pronounced effect on the desorption efficiency than variation of the desorption pressure. One also sees that the maximum recovery occurs at slightly lower desorption pressures when using higher methanol percentages. The sharp decrease in recovery with 2.7 mol% methanol-modified carbon dioxide can be explained by the LC effect described above. Fig. 4 illustrates this effect in more detail by showing the retention time of pyrazophos on the analytical column as a function of the desorption pressure for the two methanol percentages in Fig. 3. With 2.7 mol% methanol the retention time of pyrazophos on the analytical column drops immediately on increasing the desorption pressure, but with 0.6 mol% methanol it remains constant up to 140 bar. The other pesticides showed a similar dependence of the desorption efficiency and the retention time on the desorption pressure.

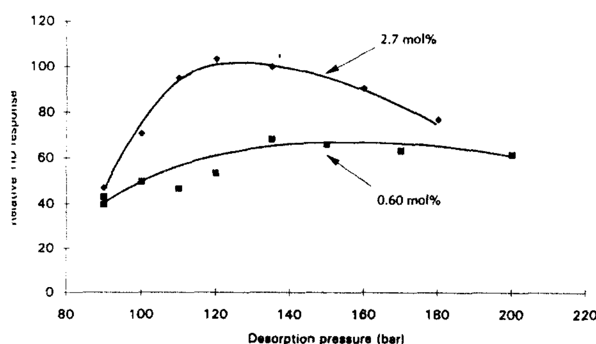

Fig. 3. Influence of the desorption pressure on the recovery of pyrazophos for (■) for 0.60 and (◆) 2.7 mol% methanol-modified carbon dioxide; response (peak area) for 135 bar and 2.7 mol-% is 100%; for each point $n = 2$. Injected amount, 75.1 μl of 10 μg l^{-1} pyrazophos; for each point $n = 2$. Desorption with 200 μl of carbon dioxide at 60 μl min^{-1}. Other conditions as in Fig. 2.

Fig. 5 shows the desorption efficiency of azinphos-methyl as a function of the desorption flow-rate. Because the influence of the desorption flow-rate is modest, one can conclude that mass transfer in the precolumn is rapid enough even at the maximum flow-rate used. This is interesting as it opens up the possibility of using larger precolumns. The lower desorption efficiency at low desorption flow-rates can be explained by the longer time necessary for desorption. During

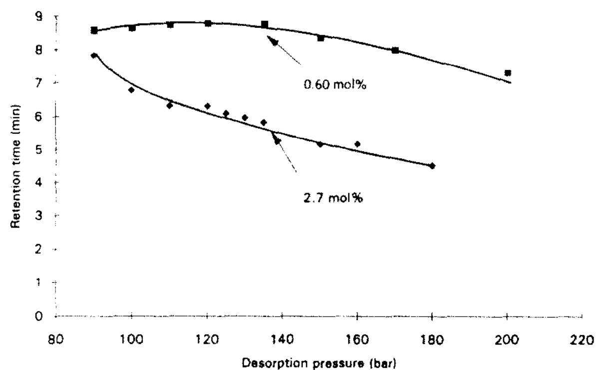

Fig. 4. Influence of the desorption pressure on the retention time of pyrazophos for (■) 0.60 and (◆) 2.7 mol-% methanol-modified carbon dioxide. Conditions as in Fig. 3.

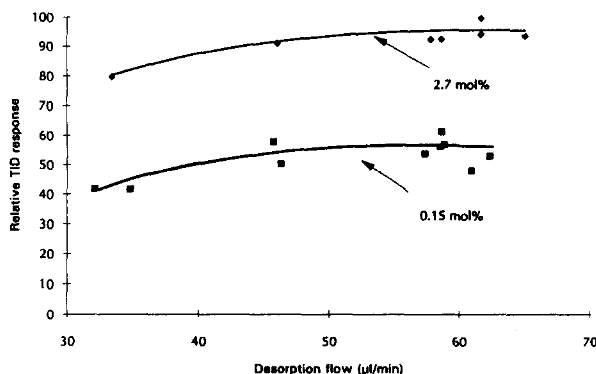

Fig. 5. Influence of the desorption flow-rate (as determined by the restrictor dimensions and calculated by dividing the desorption volume by the desorption time) on the recovery of azinphos-methyl for (■) 0.15 and (◆) 2.7 mol% methanol-modified carbon dioxide; response (peak area) for 60 μl min^{-1} and 2.7 mol-% is 100%; for each point $n = 2$. Injected amount, 75.1 μl of 15 μg l^{-1} azinphos-methyl. Desorption with 200 μl of carbon dioxide at 150 bar. Other conditions as in Fig. 2.

this time the analytes will diffuse axially in the analytical column, which will cause peak broadening.

Fig. 6 shows the dependence of the desorption efficiency on the desorption volume with pure carbon dioxide and carbon dioxide containing 1.1 mol% methanol as the desorption fluid, and with diazinon as test solute. In both instances a plateau is reached, but for the modified carbon dioxide this plateau is distinctly higher than for pure carbon dioxide. The results also indicate that the actual desorption volume used is of less importance than the amount of modifier added. For the other OPPs studied the effect of methanol addition was even more dramatic: for pyrazophos the recovery with 1.1 mol% methanol was six times higher than that without methanol, and for azinphos-methyl the gain was more than one order of magnitude.

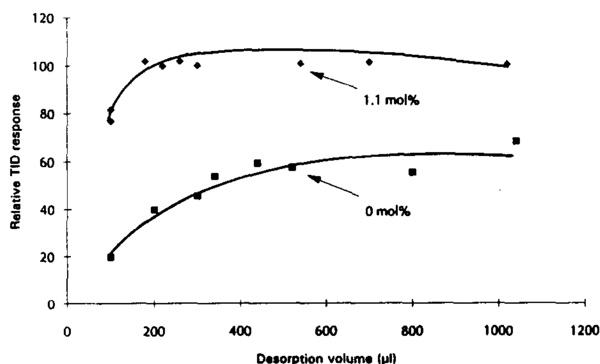

Fig. 6. Influence of the desorption volume on the recovery of diazinon for (■) 0 and (◆) 1.1 mol-% methanol-modified carbon dioxide; response (peak area) for 150 bar, 300 μl with 1.1 mol% methanol modified carbon dioxide is 100%; for each point $n = 2$. Injected amount, 75.1 μl of 5 μg l^{-1} diazinon. Desorption at 150 bar and 60 μl min^{-1}. Other conditions as in Fig. 2.

Finally, in this study two SFC pumps were normally used in order to be able to vary the modifier percentage of the fluid used for desorption without having to change the mobile phase used for the analytical separation. However, as is shown in Fig. 4, there is a difference in retention time for different amounts of modifier in the desorption fluid although the mobile phase used in the analytical column is the same. This obviously is the result of the fact that all the desorption fluid is flushed through the analytical column. Actually, apart from influencing retention times, the use of two different mobile phases will also diminish the reproducibility of the experiments. However, as was demonstrated above (*cf.*, Fig. 2), it is not necessary to use a desorption fluid having a different composition to the mobile phase if the mobile phase contains enough modifier. Therefore, it was decided to carry out the testing of the analytical performance of the system with only one SFC pump containing 1.2 mol% methanol. With this mobile phase composition desorption of the three pesticides was optimum when a desorption pressure of 150 bar, a desorption volume of 300 μl and a desorption flow-rate of 60 μl min^{-1} were chosen. An injection volume of 75 μl of aqueous sample was used to load the precolumn.

3.3. *Analytical performance*

In order to calculate the absolute recovery of the compounds, the peak areas of direct injections of a 5–15 mg l^{-1} solution of the OPPs in hexane were compared with those of 15–45 μg l^{-1} solutions of pesticide in water–methanol

(85:15, v/v) when these were analysed by means of SFE–SFC–TID, using 75-μl sample volumes. The calculated recoveries varied from 106 ± 5% ($n = 6$) for diazinon via 112 ± 7% ($n = 6$) for azinphos-methyl to 140 ± 11% ($n = 6$) for pyrazophos. One problem is that hexane is not an ideal solvent for these pesticides and causes some peak distortion and analyte losses due to adsorption on surfaces. This probably explains the high recoveries found with the on-line trace enrichment approach. The repeatability of the procedure varied from 4 to 6% ($n = 8$, 5–15 μg l^{-1}) and the reproducibility was 5–8% ($n = 2 \times 8$, 5–15 μg l^{-1}). These values are significantly better than those found with direct SFC–TID (see above). This may be due to the fact that the large-volume injections cause a decrease in the relative volume error, and/or to the disturbance caused by the introduction of hexane (see above).

In Table 2 the analytical data for the determination of the pesticides in spiked river Meuse samples are given. The calibration graphs are linear over two orders of magnitude. Fig. 7 shows SPE–SFC–TID traces for a river Meuse sample spiked with the pesticides at the 3–9 μg l^{-1} level, a river Meuse blank and a distilled water blank. In all instances 75-μl water samples were processed. As the breakthrough volumes of the analytes are higher than 10 ml (see above), 3 ml were loaded on to the precolumn. This improved the limits of detection for diazinon and azinphos-methyl to 8 and 50 ng l^{-1}, respectively

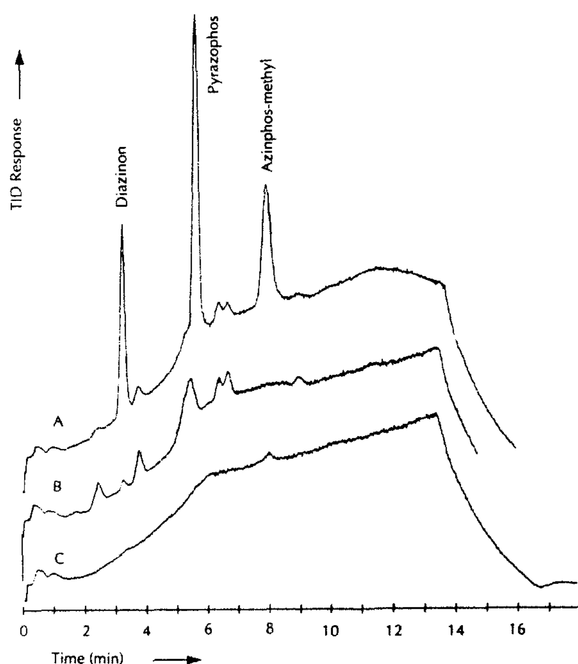

Fig. 7. SPE–SFC–TID of (A) methanol–river Meuse water (15:85, v/v) spiked with 3 μg l^{-1} of diazinon, 6 μg l^{-1} of pyrazophos and 9 μg l^{-1} azinphos-methyl, (B) methanol–river Meuse water (15:85, v/v) blank and (C) methanol–demineralized water (15:85, v/v). Injected amount, 75.1 μl; precolumn, 2.0 × 1.0 mm I.D. packed with 40-μm Bondesil C_{18}. Desorption with 300 μl of carbon dioxide modified with 1.2 mol% methanol at 150 bar and 60 μl min^{-1}. SFC conditions as in Fig. 2.

(Table 3). Because a small (unidentified) peak showed up in SPE–SFC–TID of the blank surface water sample close to pyrazophos, the

Table 2
Calibration data for three organophosphorus pesticides in spiked river Meuse water

Analyte	Limit of detection[a] (μg l^{-1})	Largest amount injected (μg l^{-1})	Calibration graph[b]	
			$y = a(\sigma_a)x + b(\sigma_b)$[c]	R^2
Diazinon	0.2	100	$y = 52.3\,(1.0)x - 42.7\,(40.4)$	0.997
Pyrazophos	1.8	200	$y = 40.4\,(2.1)x - 46.5\,(110)$	0.989
Azinphos-methyl	0.5	300	$y = 19.8\,(0.9)x - 12.5\,(10.6)$	0.987

Injected amount, 75 μl; precolumn, 2.0 × 1.0 mm I.D. packed with 40-μm Bondesil C_{18}. Desorption with 300 μl carbon dioxide modified with 1.2 mol-% methanol at 150 bar and 60 μl min^{-1}. For SFC conditions, see Table 1.
[a] Signal-to-noise ratio = 3.
[b] Eight data points in duplicate.
[c] Taken from limit of detection to largest amount injected.

Table 3
Limits of detection found with different sample introduction methods

Method of sample introduction	Sample volume	Sample solvent	Limit of detection[a] ($g\ l^{-1}$)		
			Diazinon	Pyrazophos	Azinphos-methyl
Direct (internal) loop injection	100 nl	Hexane	$0.3 \cdot 10^{-3}$	$0.6 \cdot 10^{-3}$	$1.0 \cdot 10^{-3}$
Loop injection + precolumn (2.0 × 1.0 mm I.D.)	75 μl	Methanol–river water (15:85)	$0.2 \cdot 10^{-6}$	$1.8 \cdot 10^{-6}$ [b]	$0.5 \cdot 10^{-6}$
Sample pump + precolumn (2.0 × 1.0 mm I.D.)	3 ml	Methanol–river water (15:85)	$8 \cdot 10^{-9}$	$1.8 \cdot 10^{-6}$ [b]	$50 \cdot 10^{-9}$
Sample pump + precolumn (10.0 × 2.0 mm I.D.)	47 ml	Methanol–river water (15:85)	$50 \cdot 10^{-12}$	$1.8 \cdot 10^{-6}$ [b]	$400 \cdot 10^{-12}$

[a] Desorption with 300 μl of carbon dioxide modified with 1.2 mol-% methanol at 150 bar and 60 μl min^{-1}. For SFC conditions, see Table 1.
[b] Matrix inference.

detection limit for this OPP was higher, *viz.*, 1.8 μg l^{-1}.

As mentioned above, the amount of modifier is the critical parameter to be dealt with during preconcentration. It was decided, therefore, to try a larger precolumn, *i.e.*, 10 × 2.0 mm I.D. The sample volume was increased correspondingly and 47 ml of river Meuse water were now used for on-line SPE–SFC–TID. The chromatogram for a sample spiked with 150–450 pg l^{-1} of the three pesticides is shown in Fig. 8. As can be seen, both diazinon and azinphos-methyl can still be detected, but pyrazophos has been lost owing to co-elution with some other peaks. In this instance, the limits of detection (see Table 3) were very good, *viz.*, 50 pg l^{-1} for diazinon and 400 pg l^{-1} for azinphos-methyl.

4. Conclusions

With the present on-line SPE–SFC system it is possible to enhance analyte detectability about 1000-fold compared with systems using conventional injection. The analytical data such as repeatability, reproducibility and linearity are fully satisfactory. The LC-type precolumn used for the SPE step shows well defined behaviour during both sorption and desorption. It can be used at room temperature, which is a distinct advantage when working with thermolabile compounds. The low desorption pressures that can be used when modified carbon dioxide is selected as the mobile phase help to increase the lifetime of the precolumn. Actually, in this study the precolumns were replaced, merely as a precaution, once a week. On opening them, no holes were observed; obviously the pressure changes did not cause any damage.

With the SPE–SFC–TID set-up described, organophosphorus pesticides can easily be detected and determined at levels far below the threshold values of 0.1–1 μg l^{-1} set by the European Community for drinking and surface waters. In other words, the system can be recommended for the determination of pesticides and related environmental contaminants that are too polar to be determined by means of capillary GC, or for which LC combined with diode-array UV detection is not appropriate because of a lack of chromophoric groups. Another interesting aspect of the system is that the on-line set-up will facilitate automation, an option which is, of course, not restricted to the use of TID. Finally,

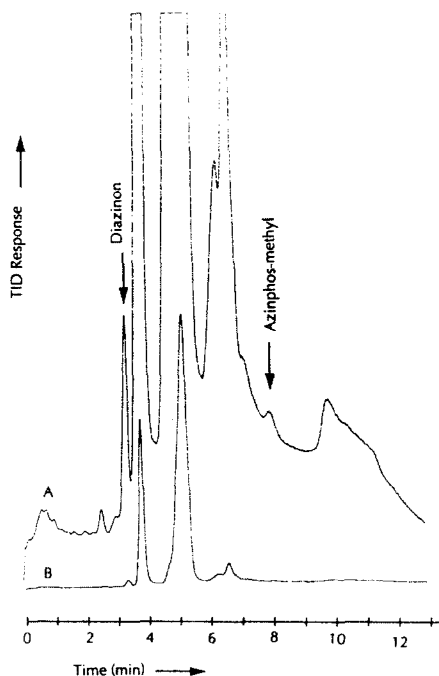

Fig. 8. Chromatogram of a large-volume injection with the SPE–SFC–TID system for methanol–river Meuse water (15:85, v/v) spiked with 150 pg l^{-1} of diazinon, (300 pg l^{-1} of pyrazophos) and 450 pg l^{-1} of azinphos-methyl at two different attenuations [(A) × 32; (B) × 1]. Injected amount, 46.8 ml; precolumn, 10 × 2.0 mm I.D. packed with 40-μm Bondesil C_{18}. Desorption with 300 μl of carbon dioxide modified with 1.2 mol% methanol at 150 bar and 60 μl min^{-1}. SFC conditions as in Fig. 2.

it should be emphasized that the precolumn-based approach allows the direct introduction of even large aqueous samples and thus simplifies sample pretreatment. The preliminary results on the use of 10-mm long precolumns are encouraging, with limits of detection in the sub-ng l^{-1} range. Although our experience is at present limited and further work will consequently have to be carried out in the application area, it seems fair to state that the gain in analyte detectability offered by the present system opens up new perspectives for SFC as a separation technique for trace-level analysis.

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